Pitpna Cas9-CKO Strategy
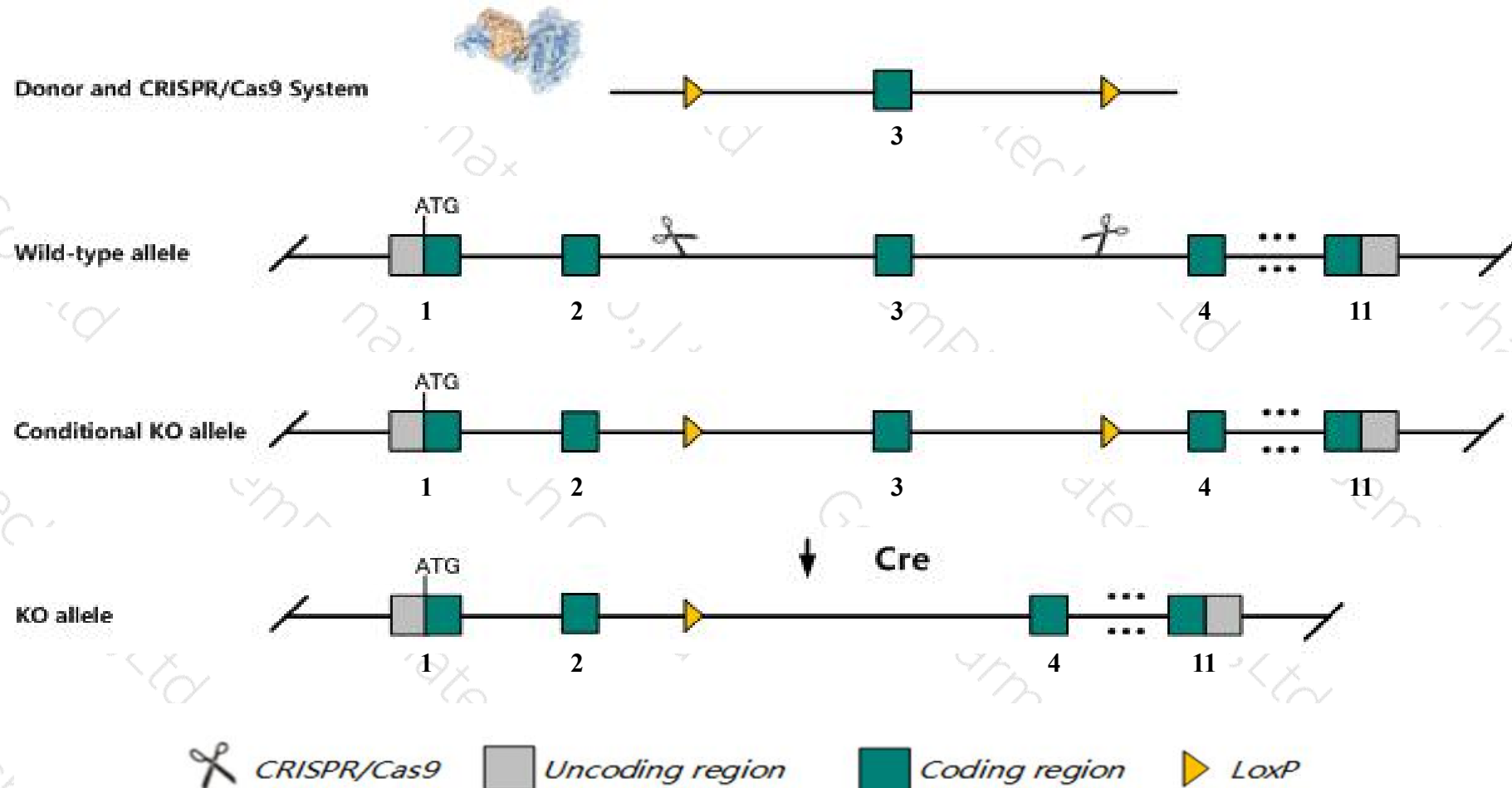
Designer: Lingyan Wu

Reviewer: Rui   Xiong

Design Date: 2020-5-20

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pitpna* gene. The schematic diagram is as follows:

Technical routes

➢ The *Pitpna* gene has 6 transcripts. According to the structure of *Pitpna* gene, exon3 of *Pitpna-203* (ENSMUST00000143219.7) transcript is recommended as the knockout region.The region contains Ens 149bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pitpna* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6J mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6J mice.

➢ The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ According to the existing MGI data,mutations of this gene result in motor coordination abnormalities and early death.

➢ The floxed region is near to the N-terminal of *4931413K12Rik* gene,this strategy may influence the regulatory function of the N-terminal of *4931413K12Rik* gene.

➢ The *Pitpna* gene is located on the Chr11. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

➢ This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

Pitpna phosphatidylinositol transfer protein, alpha [Mus musculus (house mouse)]

Gene ID: 18738, updated on 13-Mar-2020

▲ Summary

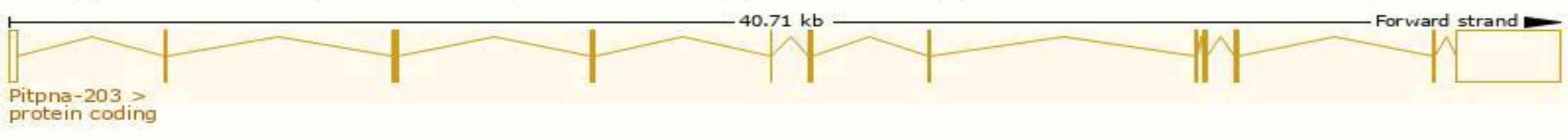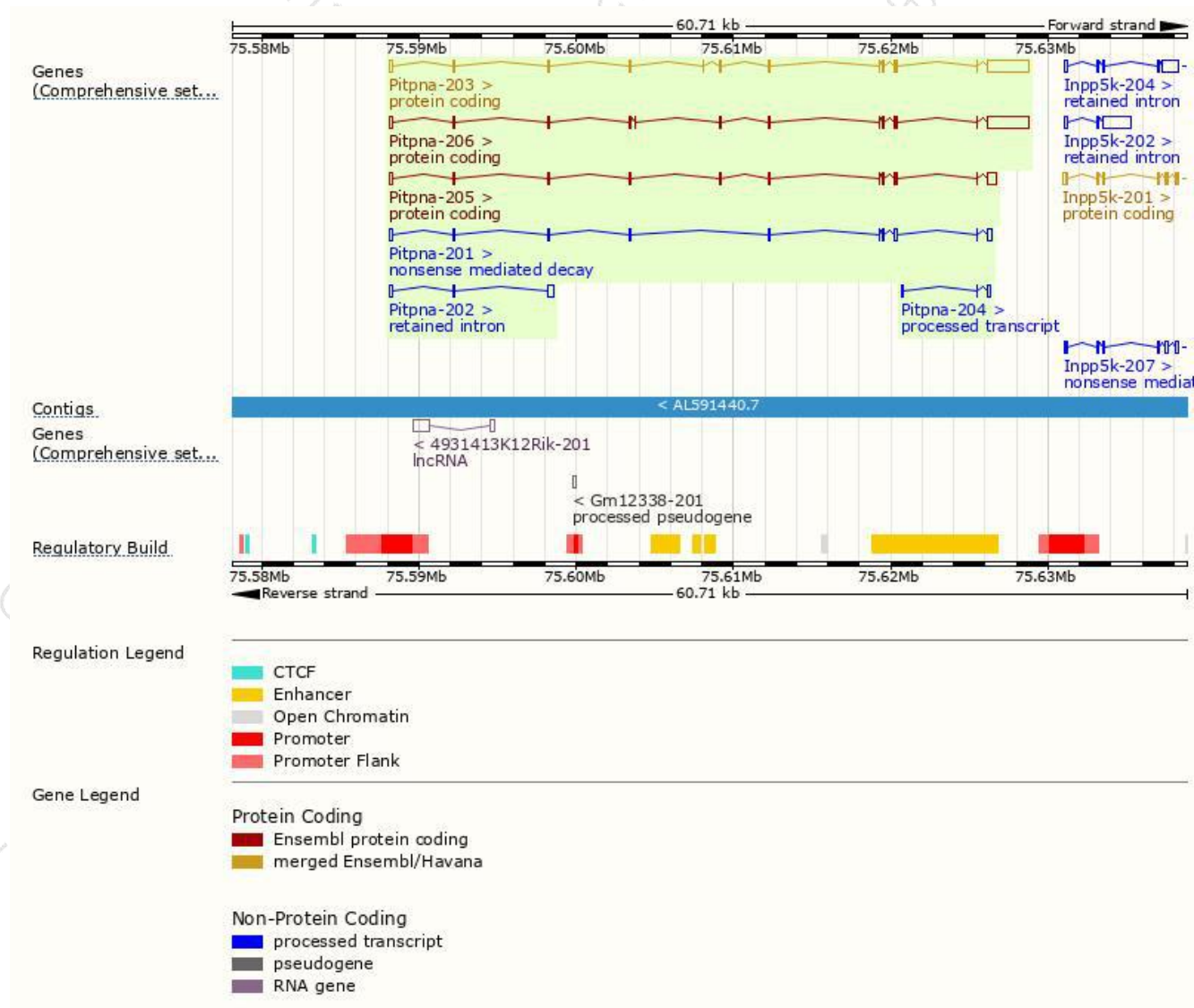|	
Official Symbol	Pitpna provided by MGI
Official Full Name	phosphatidylinositol transfer protein, alpha provided by MGI
Primary source	MGI:MGI:99887
See related	Ensembl:ENSMUSG00000017781
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Pitpn, vb, vibrator
Summary	This gene encodes a member of a family of lipid-binding proteins that transfer molecules of phosphatidylinositol or phosphatidylcholine between membrane surfaces. The protein is implicated in phospholipase C signaling and in the production of phosphatidylinositol 3,4,5-trisphosphate by phosphoinositide-3-kinase. [provided by RefSeq, Sep 2015]
Expression	Ubiquitous expression in cerebellum adult (RPKM 61.1), cortex adult (RPKM 50.1) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 6 transcripts,all transcripts are shown below:

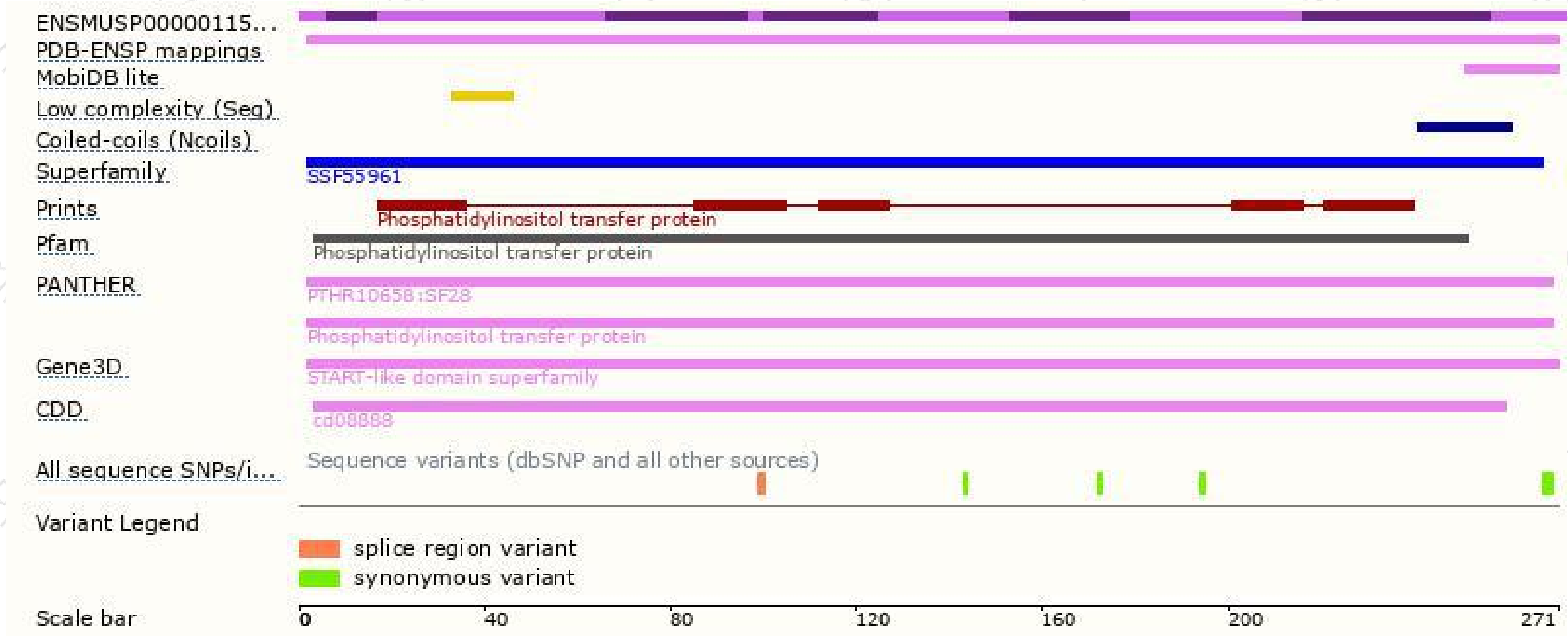| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pitpna-203	ENSMUST00000143219.7	3761	271aa	Protein coding	CCDS25052	P53810 Q5ND42	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Pitpna-206	ENSMUST00000179521.7	3727	272aa	Protein coding	-	J3QPW1	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Pitpna-205	ENSMUST00000179445.7	1665	271aa	Protein coding	-	J3QQ30	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Pitpna-201	ENSMUST00000102509.10	1245	99aa	Nonsense mediated decay	-	F8WGG5	TSL:5
Pitpna-204	ENSMUST00000153768.1	384	No protein	Processed transcript	-	-	TSL:2
Pitpna-202	ENSMUST00000140841.1	594	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Pitpna-203* transcript,the transcription is shown below:
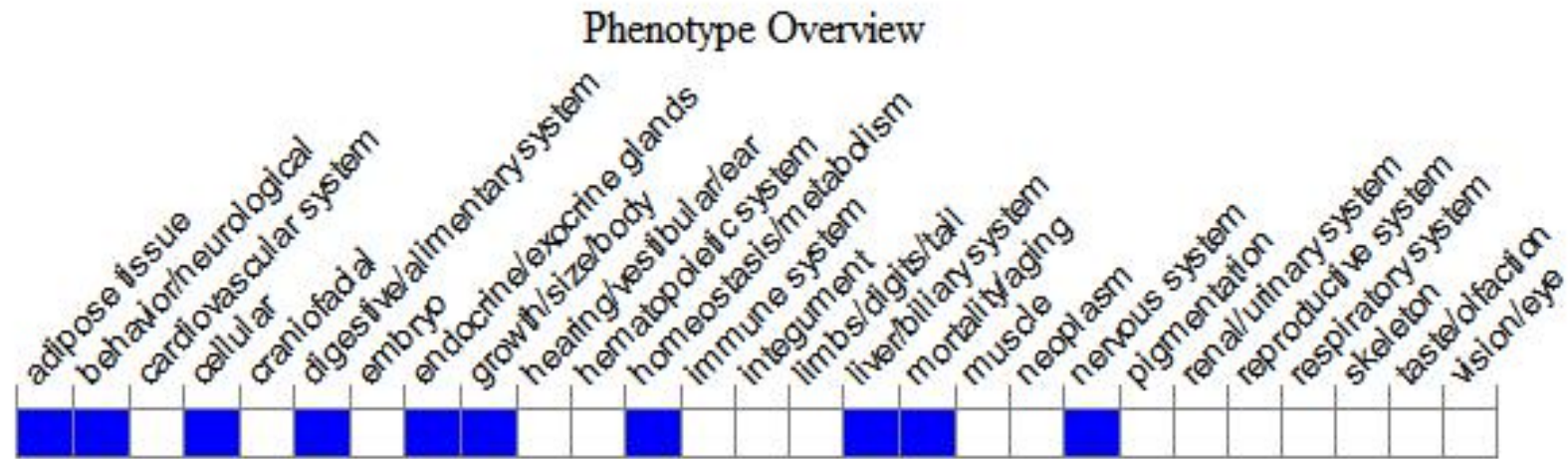
Genomic location distribution

Protein domain

ENSMUSP00000115...		
PDB-ENSP mappings		
MobiDB lite		
Low complexity (Seg)		
Coiled-coils (Ncoils)		
Superfamily	SSF55961	
Prints	Phosphatidylinositol transfer protein	
Pfam	Phosphatidylinositol transfer protein	
PANTHER	PTHR10658:SF28	
	Phosphatidylinositol transfer protein	
Gene3D	START-like domain superfamily	
CDD	cd08888	
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)	
Variant Legend		

splice region variant
synonymous variant

Scale bar	0	40	80	120	160	200	271

Mouse phenotype description(MGI)

Phenotype Overview

Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data,mutations of this gene result in motor coordination abnormalities and early death.

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd